

## Two New C<sub>20</sub>-Diterpenoid Alkaloids from *Aconitum carmichaelii*

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Two new C<sub>20</sub>-diterpenoid alkaloids, named aconicarchamines A and B (**1** and **2**, resp.), were isolated from *Aconitum carmichaelii*. By UV, IR, MS, and 1D- and 2D-NMR analyses, their structures were elucidated as 14,17-dihydro-14,17-dihydroxyajabicine and 15-O-acetyllassiocarpine. Compound **1** is the third C<sub>20</sub>-diterpenoid alkaloid with the lycocine skeleton bearing an exocyclic C-atom at C(14).

**Introduction.** – *Aconitum carmichaelii* DEBEAUX (Ranunculaceae), a traditional Chinese medicine known as ‘Fu-Zi’ in Chinese, is widely distributed in Yunnan, Shaanxi, and Sichuan Provinces in China, and has long been used as an analgesic and anti-inflammatory agent [1]. Previous phytochemical investigations on this plant revealed that C<sub>19</sub>- and C<sub>20</sub>-diterpenoid alkaloids were the main constituents [2–9]. Pharmacological studies demonstrated that diterpenoid alkaloids were the effective components in the *Aconitum* genus [10]. To find more biologically active substances, the roots of *A. carmichaelii* were phytochemically investigated to afford two new C<sub>20</sub>-diterpenoid alkaloids, named aconicarchamines A<sup>1)</sup> and B<sup>1)</sup> (**1** and **2**, resp.; see Fig. 1), together with the nine known alkaloids talatisamine, benzoylmesaconitine, fuziline, karakoline, neoline, songorine, hyaconitine, mesaconitine, and aconitine. We now report on the isolation and structure elucidation of the two new alkaloids.

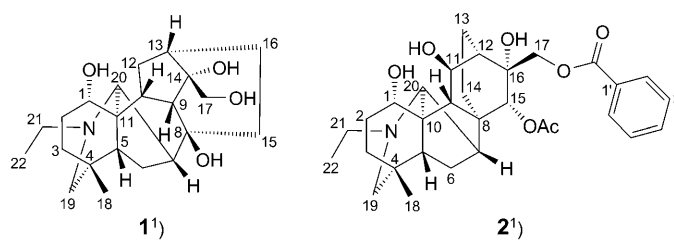


Fig. 1. Alkaloids **1** and **2**, isolated from *Aconitum carmichaelii*

<sup>1)</sup> Trivial atom numbering; for systematic names, see *Exper. Part*.

**Results and Discussion.** – Aconicarchamine A (**1**) was obtained as colorless prisms from  $\text{CHCl}_3$  and assigned the molecular formula  $\text{C}_{22}\text{H}_{35}\text{NO}_4$  by analyses of its FAB-MS ( $m/z$  378 ( $[M + \text{H}]^+$ )) and HR-ESI-MS ( $m/z$  378.2635 ( $[M + \text{H}]^+$ )). The IR spectrum showed an absorption band for OH groups ( $3443\text{ cm}^{-1}$ ). In the  $^1\text{H-NMR}$  spectrum (Table), two Me signals at  $\delta(\text{H})$  0.84 (*s*, Me(18)) and 1.08 (*t*,  $J = 7.0\text{ Hz}$ ,  $\text{MeCH}_2\text{-N}$ ) were observed, together with an O-bearing  $\text{CH}_2$  group ( $\delta(\text{H})$  3.42 and 3.45 ( $2d$ ,  $J = 11.5\text{ Hz}$ , each 1 H,  $\text{CH}_2(17)$ )). Its  $^{13}\text{C-NMR}$  (DEPT) revealed the presence of 22 C-atoms (Table) including two Me, nine  $\text{CH}_2$ , and seven CH groups, besides four quaternary C-atoms. Detailed analyses of the MS and 1D-NMR spectra suggested that compound **1** was a  $\text{C}_{20}$ -diterpenoid alkaloid. Careful comparison of the 1D-NMR data of **1** with those of ajabicine [11] suggested that they share a similar skeleton, except for the O-bearing  $\text{CH}_2(17)$  group ( $\delta(\text{C})$  66.5;  $\delta(\text{H})$  3.42 and 3.45) and a different

Table.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR Data ( $\text{CDCl}_3$ , 400 and 100 MHz, resp.) of Compounds **1** and **2**.  $\delta$  in ppm,  $J$  in Hz.

	<b>1</b>		<b>2</b>	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$
H-C(1)	3.69 ( <i>d</i> , $J = 5.3$ )	72.3 ( <i>d</i> )	4.75 ( <i>dd</i> , $J = 10.5, 6.0$ )	70.1 ( <i>d</i> )
$\text{CH}_2(2)$	1.53–1.59, 1.59–1.66 ( $2m$ )	29.5 ( <i>t</i> )	1.85–1.92, 1.97–2.04 ( $2m$ )	30.6 ( <i>t</i> )
$\text{CH}_2(3)$	1.44–2.01, 1.66–1.71 ( $2m$ )	31.0 ( <i>t</i> )	1.25–1.32, 1.43–1.50 ( $2m$ )	38.4 ( <i>t</i> )
C(4)		32.8 ( <i>s</i> )		33.5 ( <i>s</i> )
H-C(5)	1.91 ( <i>d</i> , $J = 7.3$ )	46.2 ( <i>d</i> )	1.42 ( <i>d</i> , $J = 7.0$ )	52.7 ( <i>d</i> )
$\text{CH}_2(6)$	1.81 ( <i>dd</i> , $J = 14.2, 7.2$ ), 2.14 ( <i>dd</i> , $J = 14.2, 7.3$ )	24.9 ( <i>t</i> )	1.21–1.29 ( <i>m</i> ), 3.52 ( <i>dd</i> , $J = 12.2, 7.0$ )	23.0 ( <i>t</i> )
H-C(7)	1.75 ( <i>d</i> , $J = 6.8$ )	44.0 ( <i>d</i> )	2.23 ( <i>br. s</i> )	42.0 ( <i>d</i> )
C(8)		78.4 ( <i>s</i> )		42.8 ( <i>s</i> )
H-C(9)	1.98 ( <i>d</i> , $J = 7.2$ )	48.8 ( <i>d</i> )	2.41 ( <i>d</i> , $J = 9.0$ )	51.4 ( <i>d</i> )
H-C(10) or C(10)	1.58 ( <i>dd</i> , $J = 7.2, 7.0$ )	46.7 ( <i>d</i> )		51.3 ( <i>s</i> )
C(11) or H-C(11)		48.7 ( <i>s</i> )	5.46 ( <i>d</i> , $J = 9.0$ )	70.3 ( <i>d</i> )
$\text{CH}_2(12)$ or H-C(12)	1.44–1.51, 2.20–2.27 ( $2m$ )	35.4 ( <i>t</i> )	2.56 ( <i>br. s</i> )	45.2 ( <i>d</i> )
H-C(13) or $\text{CH}_2(13)$	2.04 ( <i>d</i> , $J = 9.5$ )	35.7 ( <i>d</i> )	1.42–1.49, 2.29–2.36 ( $2m$ )	20.1 ( <i>t</i> )
C(14) or $\text{CH}_2(14)$		82.7 ( <i>s</i> )	0.95–0.99, 1.72–1.79 ( $2m$ )	27.4 ( <i>t</i> )
$\text{CH}_2(15)$ or H-C(15)	1.95 ( <i>dd</i> , $J = 9.5, 3.5$ ), 1.70–1.75 ( <i>m</i> )	33.1 ( <i>t</i> )	4.68 ( <i>d</i> , $J = 4.0$ )	85.9 ( <i>d</i> )
$\text{CH}_2(16)$ or C(16)	1.64–1.69, 1.77–1.84 ( $2m$ )	32.7 ( <i>t</i> )		77.3 ( <i>s</i> )
$\text{CH}_2(17)$	3.42, 3.45 ( $2d$ , $J = 11.5$ )	66.5 ( <i>t</i> )	5.32, 5.56 ( $2d$ , $J = 11.5$ )	69.3 ( <i>t</i> )
Me(18)	0.84 ( <i>s</i> )	27.5 ( <i>q</i> )	0.68 ( <i>s</i> )	25.8 ( <i>q</i> )
$\text{CH}_2(19)$	2.03, 2.23 ( $2d$ , $J = 11.0$ )	60.3 ( <i>t</i> )	2.17, 2.51 ( $2d$ , $J = 11.0$ )	56.7 ( <i>t</i> )
H-C(20)	3.03 ( <i>s</i> )	63.1 ( <i>d</i> )	4.08 ( <i>s</i> )	66.9 ( <i>d</i> )
$\text{MeCH}_2\text{-N}$	2.48–2.56, 2.37–2.44 ( $2m$ )	48.1 ( <i>t</i> )	2.42–2.46, 2.32–2.37 ( $2m$ )	50.7 ( <i>t</i> )
$\text{MeCH}_2\text{-N}$	1.08 ( <i>t</i> , $J = 7.0$ )	13.0 ( <i>q</i> )	0.99 ( <i>t</i> , $J = 7.0$ )	13.2 ( <i>q</i> )
AcO-C(15)			1.98 ( <i>s</i> )	171.8 ( <i>s</i> ), 21.2 ( <i>q</i> )
PhCOO-C(17)				166.6 ( <i>s</i> )
C(1')				131.6 ( <i>s</i> )
H-C(2',6')			8.27 ( <i>d</i> , $J = 7.4$ )	130.1 ( <i>d</i> )
H-C(3',5')			7.28 ( <i>t</i> , $J = 7.6$ )	128.7 ( <i>d</i> )
H-C(4')			7.45 ( <i>t</i> , $J = 7.4$ )	133.0 ( <i>d</i> )

quaternary C(14) ( $\delta(\text{C})$  82.7) in **1**, instead of the C(14) bearing an exocyclic  $\text{CH}_2$  group in ajabicine; this was further confirmed by the cross-peaks  $\text{CH}_2(17)/\text{C}(14)$ ,  $\text{H}-\text{C}(9)/\text{C}(14)$ , and  $\text{H}-\text{C}(13)/\text{C}(14)$  in the HMBC spectrum (Fig. 2).

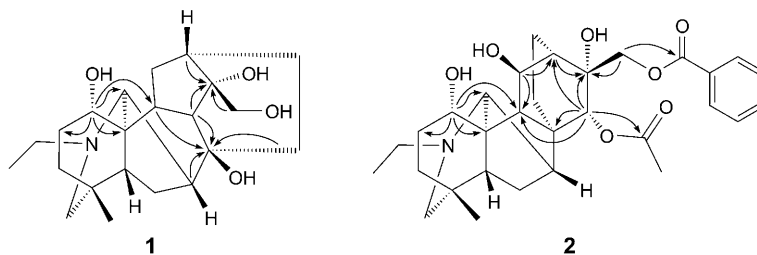


Fig. 2. Selected HMBCs ( $\text{H} \rightarrow \text{C}$ ) of alkaloids **1** and **2**

Compound **1** was shown to possess the same relative configuration as ajabicine, this being supported not only by their almost identical  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data (Table), but also by the ROESY data (Fig. 3) showing correlations between  $\text{H}-\text{C}(9)$ ,  $\text{H}-\text{C}(13)$  (assumed to be  $\beta$ -orientation in ajabicine), and  $\text{CH}_2(17)$ , *i.e.*,  $\beta$ -orientation of the  $\text{OH}-\text{CH}_2(17)$  substituent. Thus, the structure of compound **1** was determined as 14,17-dihydro-14,17-dihydroxyajabicine<sup>1</sup>, named aconicarchamine A (**1**).

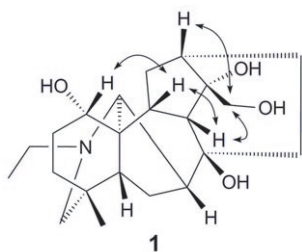


Fig. 3. Selected ROESY correlations of alkaloid **1**

Aconicarchamine B (**2**) was isolated as a white powder. The molecular formula was determined to be  $\text{C}_{31}\text{H}_{41}\text{NO}_7$  based on the FAB-MS ( $m/z$  540 ( $[\text{M} + \text{H}]^+$ )) and HR-FAB-MS ( $m/z$  540.2965 ( $[\text{M} + \text{H}]^+$ )). The IR spectrum showed absorption bands for OH groups ( $3444 \text{ cm}^{-1}$ ), a conjugated ester CO group ( $1721 \text{ cm}^{-1}$ ), and for an aromatic ring ( $1603$ ,  $1550$ , and  $1451 \text{ cm}^{-1}$ ). The  $^1\text{H}$ -NMR data revealed the presence of an EtN group ( $\delta(\text{H})$  0.99 (*t*,  $J = 7.0 \text{ Hz}$ ,  $\text{MeCH}_2-\text{N}$ )), a quaternary Me group ( $\delta(\text{H})$  0.68 (*s*, Me(18))), an AcO ( $\delta(\text{H})$  1.98 (*s*, 3 H)), and a benzoyl group ( $\delta(\text{H})$  8.27 (*d*,  $J = 7.4 \text{ Hz}$ , 2 H), 7.45 (*t*,  $J = 7.4 \text{ Hz}$ , 1 H), 7.28 (*t*,  $J = 7.6 \text{ Hz}$ , 2 H)), and the absence of MeO or  $\text{O}-\text{CH}_2-\text{O}$  groups. These features are characteristic of an atisine-type  $\text{C}_{20}$ -diterpenoid alkaloid bearing an EtN group. Comparison of the 1D-NMR spectra (Table) of **2** with those of lassiocarpine [12] showed high similarity except that there was an additional AcO group ( $\delta(\text{H})$  1.98 (*s*);  $\delta(\text{C})$  171.8 (*s*); and 21.2 (*q*)) in compound **2**. The AcO group was determined to be linked to C(15) by the HMBCs between  $\delta(\text{H})$  4.68 (*d*,  $J = 4.0 \text{ Hz}$ ,

H–C(15)) and  $\delta(\text{C})$  171.8 (AcO, C=O), C(8), C(9), C(12), and C(16). The 2D-NMR spectra including HMQC, HMBC, COSY, and ROESY resulted in the assignments of all H- and C-atoms of compound **2** (Table). Accordingly, aconicarchamine B (**2**) was established to be 15-*O*-acetyllassiocarpine.

As far as we know, ajabicine [11] and actaline [13] are the only two lycotene-skeleton C<sub>20</sub>-diterpenoids reported before. Compound **1** is the third C<sub>20</sub>-diterpenoid with a lycotene skeleton bearing an exocyclic C-atom at C(14) isolated from a natural source, providing a new candidate for further pharmacological investigation.

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### Experimental Part

*General.* Column chromatography (CC): silica gel (SiO<sub>2</sub>; 200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, P. R. China); Al<sub>2</sub>O<sub>3</sub> (Shanghai Wusi Chemical Reagents Company, Ltd.); Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd., Germany). M.p.: XRC-1 micro melting-point apparatus; uncorrected. Optical rotations: Jasco-DIP-370 digital polarimeter. UV Spectra: Shimadzu-UV-2401A spectrophotometer. IR Spectra: Bio-Rad-FTS-135 spectrometer. 1D- and 2D-NMR Spectra: Bruker-AM-400 and -DRX-500 spectrometers; chemical shifts  $\delta$  in ppm with reference to the solvent signals. MS: VG-Autospec-3000 spectrometer at 70 eV; in *m/z* (rel. %). HR-ESI-MS: API-Qstar-Pulsar-1 spectrometer; in *m/z*.

*Plant Material.* The roots of *Aconitum carmichaelii* DEBEAUX were collected in Hanzhong, Shaanxi Province, P. R. China, in October 2003, and authenticated by Prof. Dr. Li-Gong Lei, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. KIB 2003-11-03) has been deposited in the Group of anti-Virus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

*Extraction and Isolation.* The roots of *A. carmichaelii* (30 kg) were powdered and extracted three times with 95% EtOH for 2 h under reflux. After evaporation of the solvent, the crude extract was dissolved in 6 l of 1.5% aq. HCl soln. and the soln. then filtrated. The acidic soln. was basified to pH 9.0 with ammonia (25%) and then extracted with CHCl<sub>3</sub>. The org. phase was concentrated and the crude alkaloid extract (320 g) subjected to CC (SiO<sub>2</sub> (1.6 kg), petroleum ether/Me<sub>2</sub>CO/Et<sub>2</sub>NH 10:1:1 → 3:1:1): Fractions 1–6. Fr. 5 (15.5 g) was applied to CC (SiO<sub>2</sub> (230 g), petroleum ether/Me<sub>2</sub>CO/Et<sub>2</sub>NH 15:9:1): Frs. 5.1–5.3. Fr. 5.2 (0.9 g) was further purified by CC (SiO<sub>2</sub> (15 g), benzene/AcOEt/Et<sub>2</sub>NH 10:4:1): **1** (18 mg). Fr. 4 (20.8 g) was subjected to CC (SiO<sub>2</sub> (300 g), petroleum ether/Me<sub>2</sub>CO/Et<sub>2</sub>NH 15:8:1); then neutral Al<sub>2</sub>O<sub>3</sub> (100 g; 200–300 mesh), petroleum ether/Me<sub>2</sub>CO 4:1): **2** (20 mg).

*Aconicarchamine A* (=14,17-Dihydro-14,17-dihydroxyajabicine = (1 $\alpha$ ,14 $\alpha$ )-20-Ethyl-14-(hydroxymethyl)-4-methylaconitane-1,8,14-triol; **1**): White prisms (CHCl<sub>3</sub>). M.p. 210–211°.  $[\alpha]_{\text{D}}^{15.8} = +3.60$  ( $c = 0.36$ , MeOH). IR (KBr): 3443, 1033. NMR: Table. FAB-MS (pos.): 378 ( $[M + H]^+$ ), 361 (8), 148 (22), 122 (33), 117 (21), 105 (33), 91 (52), 79 (36), 58 (100). HR-FAB-MS (pos.): 378.2635 ( $[M + H]^+$ , C<sub>22</sub>H<sub>36</sub>NO<sub>4</sub><sup>+</sup>; calc. 378.2234).

*Aconicarchamine B* (=15-*O*-Acetyllassiocarpine = (1 $\alpha$ ,7 $\beta$ ,11 $\beta$ ,15 $\alpha$ )-15-(Acetyloxy)-21-ethyl-1,11,16-trihydroxy-4-methyl-7,20-cyclootidan-17-yl Benzoate; **2**): White power. M.p. 184–185°.  $[\alpha]_{\text{D}}^{15.2} = -11.35$  ( $c = 0.23$ , MeOH). UV (MeOH): 230 (3.85). IR (KBr): 3444, 1721, 1640, 1603, 1550, 1451, 1247, 1098, 715. NMR: Table. FAB-MS (pos.): 540 ( $[M + H]^+$  (100), 522 (17). HR-FAB-MS (pos.): 540.2965 ( $[M + H]^+$ , C<sub>31</sub>H<sub>42</sub>NO<sub>7</sub><sup>+</sup>; calc. 540.2234).

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